

# Histopathological Findings in the Rat and Hamster Respiratory Tract in a 90-Day Inhalation Study Using Fresh Sidestream Smoke of the Standard Reference Cigarette 2R1

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## Introduction

While histopathological changes in the respiratory tract of rats and hamsters in short-term and long-term inhalation studies with mainstream smoke (MS) have been reported extensively in literature, there are only three published inhalation studies on rats and rats and hamsters with sidestream smoke in which histopathology is the main end point. In these studies, the findings observed were epithelial hyperplasia and squamous metaplasia in the rostral nose of the rat at a concentration of 4 µg/L (von Meyerinck et al. 1989) and epithelial hyperplasia in the rostral nose of the rat at a concentration of 10 µg/L (Coggins et al. 1992, 1993). No findings were seen in the rat larynx. In the hamster, no histopathological changes were observed in the respiratory tract (von Meyerinck et al. 1989).

## Methods and Evaluations

### Animals and Housing

Male Sprague Dawley rats, Crj:CDBR (Charles River, Germany), and male Syrian golden hamsters, Lak:LVG(SYR) (Charles River, U.S.A.), were used, the body weight at the start of the inhalation period being approximately 200 and 80 g, respectively. The animals were housed under standardized conditions (room temperature  $22 \pm 1$  °C, relative humidity  $55 \pm 10\%$ , and light-dark cycle 14.5 hours:9.5 hours) in polycarbonate cages, type 3, with granulated dust-free wood as bedding material. Diet and drinking water were supplied ad libitum except during exposure.

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#### Animal Exposure

*wt: fresh sediment smoke (FSS)*  
The animals were nose-only exposed for 7 hours/day, 7 days/week for 90 days in glass tubes adapted to the shape of the skull at the front end and sealed with rubber stoppers at the rear end. The TPM concentrations in the FSS of 2 and 6  $\mu\text{g}/\text{L}$  are significantly above the levels reached in occupied spaces with smoking (U.S. EPA 1992). The air flow rate in the exposure chamber, cross section  $0.1 \text{ m} \times 0.1 \text{ m}$ , was  $50 \text{ L}/\text{minute}$ . Sham-exposed animals served as controls.

#### Group Size

Twenty male rats and 20 male hamsters per group were allocated to one sham exposure group and two FSS groups. Of these, 10 rats and 10 hamsters per group were kept for a 21-day postexposure period.

#### Generation of Sidestream Smoke

University of Kentucky standard reference cigarettes 2R1 were smoked on automatic 30-port positive pressure smoking machines (mean puff volume, 35 mL; puffs/cigarette, 9.8; puff frequency/cigarette, 14/min; puff duration, 2 seconds). The resulting sidestream was collected using a circular hood inside the smoking machine. The maximum age of the smoke was approximately 7 seconds. The two FSS concentrations were obtained by dilution with particle-filtered air.

#### Characterization of Test Atmosphere

Relevant analytical parameters were determined at appropriate intervals to characterize the FSS and the air used for sham exposure as well as to check the reproducibility of the FSS generation (Table 1).

#### Carboxyhemoglobin

The steady-state proportion of carboxyhemoglobin was determined at the end of daily exposure three times during the inhalation period to confirm smoke exposure. In the low- and high-FSS groups it was 1.6 and 3.7% for the rats and 2.0 and 4.5% for the hamsters, respectively.

#### Biological Parameters

The primary parameters were gross pathology and histopathology of the respiratory tract as well as morphometrical determination of the laryngeal epithelial thickness. H&E-stained paraffin sections cut at defined levels (Young

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Table 1. Concentrations of relevant analytical parameters.

Analytical parameter	FSS group	
	Low	High
TPM ( $\mu\text{g/L}$ )	2.1	6.0
CO (ppm)	9	22
Neotoline ( $\mu\text{g/L}$ )	0.6	1.3
Ammonia ( $\mu\text{g/L}$ )	1.1	2.6
Formaldehyde (ppm)	0.19	0.38
Acetaldehyde (ppm)	0.24	0.55
Acrolein (ppm)	0.03	0.07

1981, Lewis 1980; Lamb and Redd 1969) were evaluated semiquantitatively and morphometrically. Secondary parameters were in-life observations, mortality, body weight, and organ weights.

## Results

In both species, no smoke-exposure-related effects were seen for in-life observations, mortality, body weight, organ weights, and gross pathology. The histopathological findings observed in rats were as follows:

nose (nasal): reserve cell hyperplasia of the respiratory epithelium

larynx:

base of epiglottis: hyperplasia of squamous epithelium

arytenoid projections

ventral depression: hyperplasia of cuboidal epithelium floor of the larynx;

lingual metaplasia of the pseudostratified epithelium

vocal cords:

lower medial surface: hyperplasia of the squamous epithelium (Figures 1-3)

upper medial surface: squamous metaplasia of the pseudostratified epithelium (Figures 4-6)

4-vocal folds: hyperplasia of the squamous epithelium

The severity of these findings in rats was slight, and they were observed mainly in the high FSS concentration group. No smoke-exposure-related histopathological changes were observed in trachea and lungs. A dose-dependent increase in epithelial thickness in the larynx compared to sham was observed at the floor of the larynx and at the lower medial surface of the vocal cord; the increase at the respective sites was 19 and 32% in the 6  $\mu\text{g/L}$  TPM

4: feeding should  
follow diet  
alt: colon

4: to live up  
the base and  
4: medial

4

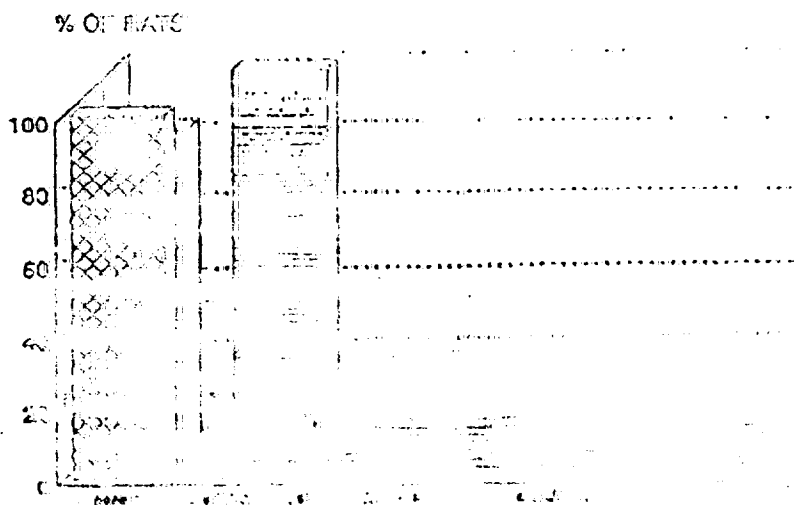


Fig. 1

concentration

findings were

etc.

reason

adverse

effect

### Conclusion

The observed effects of the treatment of the rats of the first group (cross-hatch) and the rats of the second group (dotted) have been reported. The results of the study have been considered to be significant and to be of importance.

The NOEL for the treatment of the rats of the first group (cross-hatch) is 1.0 mg/kg/day. The NOEL for the treatment of the rats of the second group (dotted) is 2.0 mg/kg/day. The NOEL for the treatment of the rats of the third group (dotted) is 3.0 mg/kg/day.

inst:  
+ Observed Effect  
Level (NOEL)

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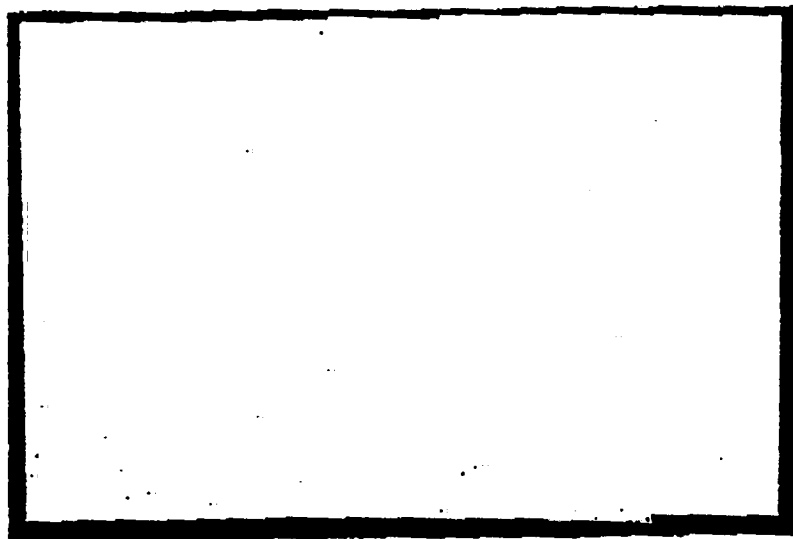


Figure 2. Transverse section at the arytenoid projections, vocal cords, lower medial surface sham-exposed rat showing normal epithelium; H & E  $\times 147$ .

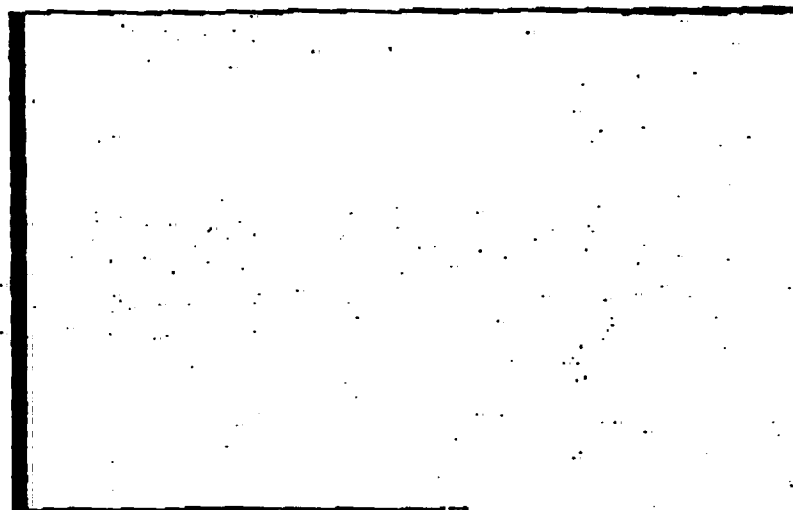


Figure 3. Transverse section at the arytenoid projections, vocal cords, lower medial surface high-dose SS-exposed rat showing hyperplasia of squamous epithelium; H & E  $\times 147$ .

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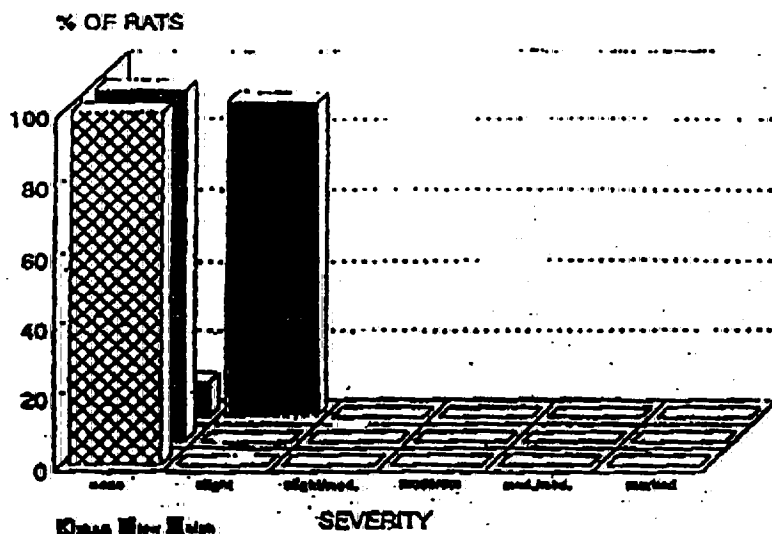


Figure 4. Distribution of squamous metaplasia at the arytenoid projections, vocal cords, upper medial surface, at the end of a 90-day inhalation period.

**Acknowledgement:** This work was sponsored by Philip Morris, U.S.A.

#### References

- Coggins CRE, Ayres PH, Mosberg AT, et al. (1992) Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. *Fundam Appl Toxicol* 19:133-140
- Coggins CRE, Ayres PH, Mosberg AT, et al. (1993) Subchronic inhalation study in rats using aged and diluted sidestream smoke from a reference cigarette. *Inhalation Toxicol* 5:77-97
- Lamb D, Reid L (1969) Goblet cell increase in rat bronchial epithelium after exposure to cigarette and cigar tobacco smoke. *Br Med J* 1: 33-35
- Lewis DJ (1980) Experimental pathology of the rat larynx following exposure to tobacco smoke [Ph.D. Thesis] University of Surrey.
- U.S. Environmental Protection Agency (1992) Respiratory health effects of passive smoking: lung cancer and other disorders. Office of Health and Environmental Assessment, Office of Research and Development, Washington, DC
- von Meyerinck L, Scherer G, Adikofer F, et al. (1989) Exposure of rats and hamsters to sidestream smoke from cigarettes in a subchronic inhalation study. *Exp Pathol* 37:186-189
- Young JT (1981) Histopathologic examination of the rat nasal cavity. *Fundam Appl Toxicol* 1:309-312

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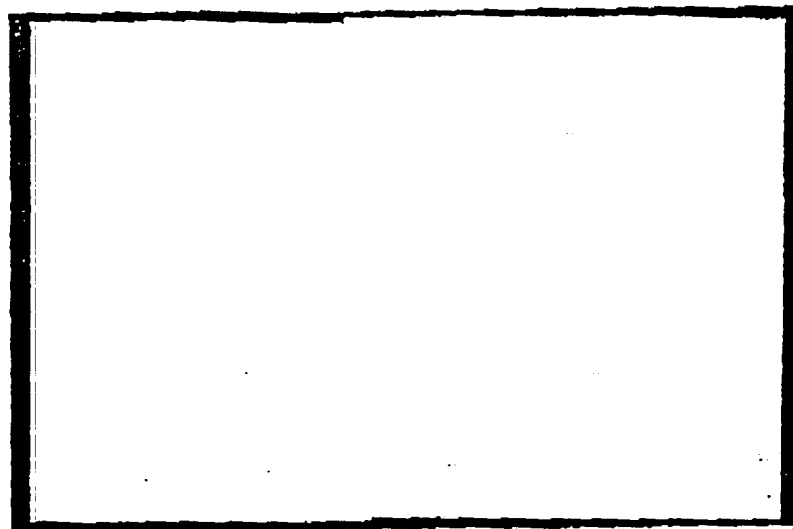


Figure 5. Transverse section at the arytenoid projections, vocal cords, upper medial surface: sham-exposed rat showing normal epithelium; H & E  $\times 147$ .



Figure 6. Transverse section at the arytenoid projections, vocal cords, upper medial surface: high-dose SS-exposed rat showing squamous metaplasia; H & E  $\times 147$ .

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